AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 1, line 19 with the following amended paragraph:

- Combinatorial libraries of synthetic and natural products are important sources of molecular information for the development of pharmacologic agents. Linear peptide libraries, containing known and random peptide sequences, are particularly good sources of new and novel compounds for drug development because of the diversity of structures which can be generated. Drawbacks to linear peptide libraries are: (1) linear peptides are generally flexible molecules with entropic limitations on achieving productive biologically active conformations; (2) linear peptides are ~~susceptibile~~ susceptible to proteolytic enzymes; and, (3) linear peptides are inherently ~~instable~~unstable. For this reason, approaches utilizing conformational and topographical constraints to restrict the number of conformational states a peptide molecule may assume have been sought. See, for example, Hruby, (1982) Life Sci., 31:189; Hruby, et al., (1990) Biochem. J. 268:249.249 (1990). -

Please replace the paragraph beginning on page 3, line 20 with the following amended paragraph:

- Figure 2A depicts intein catalyzed ligation by the Mxe GyrA intein. In ~~it's~~ the indicated configuration, intein catalyzed ligation joins the extein residues located at the junction points with each of the two intein motifs. -

Please replace the paragraph beginning on page 5, line 5 with the following amended paragraph:

- Figure 4A depicts the amino acid sequence of a modified wild-type Ssp DnaB Intein configured to generate a cyclic peptide containing a FLAG epitope. The DNA sequence is ~~provided~~ given in Figure 4B. -

Please replace the paragraph beginning on page 5, line 9 with the following amended paragraph:

- Figures 5A and B depict the nucleotide and amino acid sequence of the intein Ssp DnaB J3 template used to generate intein mutants L7-J3, E6-J3, E9-J3, C11-J3 and B8-J3, with

improved splicing efficiency. The J3 template carries a mutation which results in [a] an amino acid change D to N at position 320. Thus, all mutants based on the J3 template are double mutants. -

Please replace the paragraph beginning on page 6, line 18 with the following amended paragraph:

- Figure 12 depicts the fluorescent reporter system used to quantify intein cyclization. Figure ~~12-A~~12A depicts GFP split at the loop 3 junction and reversal of the translation order of the N- and C-terminal fragments. The termini are fused using a glycine-serine linker. The GFP is positioned within the Ssp DnaB intein ~~cyclizationscaffold~~ cyclization scaffold. Cyclized product reconstitutes both structure and fluorescence of GFP. In addition, splicing one-half of the myc epitope onto either side of the loop 3 junction allows for reconstruction of the myc epitope upon cyclization. -

Please replace the paragraph beginning on page 6, line 35 with the following amended paragraph:

- Figure 12F shows the results ~~form~~from a native gel and the ~~contribution~~signals ~~to~~from GFP fluorescence. The majority [fo]of the fluorescence arises from the formation of cyclized GFP product, bands C and D. -

Please replace the paragraph beginning on page 7, line 1 with the following amended paragraph:

- Figure 13 illustrates a functional screen for isolating randomly-generated mutants with altered cyclization activity. Figure 13A depicts a functional screen for intein mutants with ~~altered~~altered cyclization activity. Figure 13B depicts mutations modeled on the Mxe GyrA intein structure. Figure 13C depicts the sequence alignment of Mxe GyrA and Ssp DnaB inteins. Mutants are identified in shaded color. Figure 13D shows the results from a western analysis of isolated mutants. DnaB mutants E9-J3, E6-J3, C11-J3, L7-J3, and B8-J3 have cyclization efficiencies ~~were~~ are greater than the J3 starting intein template. -

Please replace the paragraph beginning on page 8, line 12 with the following amended paragraph:

- Accordingly, the present invention provides fusion ~~polypetides~~polypeptides comprising intein motifs and peptides. –

Please replace the paragraph beginning on page 9, line 19 with the following amended paragraph:

- In a preferred embodiment, the fusion polypeptide is designed with the primary sequence from the N-terminus comprising IA-target-IB. IA is defined herein as the C-terminal intein motif, IB is defined herein as the N-terminal intein motif and target is defined herein as a peptide. DNA sequences encoding the inteins may be obtained from a prokaryotic DNA sequence, such as a bacterial DNA sequence, or a eukaryotic DNA sequence, such as a yeast DNA sequence. The Intein Registry includes a list of all experimental and theoretical inteins discovered to date and submitted to the registry at world wide web neb.com/inteins/int_reg.html ~~(http://www.neb.com/inteins/int_reg.html)~~.-

Please replace the paragraph beginning on page 10, line 26 with the following amended paragraph:

- In a preferred embodiment, the fusion polypeptides of the invention comprise peptides. That is, the fusion polypeptides of the invention are translation products of nucleic acids. In this embodiment, nucleic acids are introduced into cells, and the cells express the nucleic acids to form peptides. ~~peptides.]~~ Generally, peptides ranging from about 4 amino acids in length to about 100 amino acids may be used, with peptides ranging from about 5 to about 50 being preferred, with from about 5 to about 30 being particularly preferred and from about 6 to about 20 being especially preferred. –

Please replace the paragraph beginning on page 13, line 9 with the following amended paragraph:

- In a preferred embodiment, the reporter protein is an indirectly detectable protein. As for the reporter proteins, cells that contain the indirectly detectable protein can be distinguished from those that do not; however, this is as a result of a secondary event. For example, a preferred embodiment utilizes "enzymatically detectable" reporters that comprise enzymes,

such as luciferase, β-galactosidase, and β –lactamase, ~~that~~ which will act on chromogenic, and particularly fluorogenic, substrates, to generate fluorescence~~, such as luciferase, β-galactosidase, and β-lactamase~~. Alternatively, the indirectly detectable protein may require a recombinant construct in a cell that may be activated by the reporter; for example, transcription factors or inducers that will bind to a promoter linked to an autofluorescent protein such that transcription of the autofluorescent protein occurs. –

Please replace the paragraph beginning on page 26, line 7 with the following amended paragraph:

- Preferred vectors include a vector based on the murine stem cell virus (MSCV) (see Hawley et al., Gene Therapy 1:136 (1994)) and a modified MFG virus (Rivere et al., Genetics 92:6733 (1995)), and pBABE, outlined in the examples. A general schematic of the retroviral construct is depicted in ~~Figure 6~~ Figures 6 and 15A. -

Please replace the paragraph beginning on page 28, line 27 with the following amended paragraph:

- Highly restrained cyclic peptide libraries are made by using codons which code mainly for amino acids with large side chains. That is, when several resides of a cyclic peptide encode amino acids with large side chains, the conformation space of the peptide is restricted. The result is to bias the peptide to a higher affinity by reducing peptide conformational entropy. For example, a library of cyclic peptides could be created by restricting the triplet nucleotides coding for each random amino acid in the library to ~~Cor~~ C or T for the first position of the triplet, A, G or T for the second position in the triplet, and G, C or T for the third position in the triplet. This would result in a library biased to large amino acids, i.e., phenylalanine (F), leucine (L), tyrosine (Y), histidine (H), glutamine (Q), cysteine (C), tryptophan (W) and arginine (R). A library biased toward large amino acid side chains, combined with the loss of glycine, alanine, serine, threonine, aspartate, and glutamate results in a library coding for more rigid peptides. As this library lacks an acidic amino acid, a pre-synthesized triplet coding glutamate (i.e., GAG) or aspartate (GAC) may be added during the DNA synthesis of the library. -

Please replace the paragraph beginning on page 36, line 20 with the following amended paragraph:

- In a preferred embodiment, thermocycler and thermoregulating systems are used for stabilizing the temperature of the heat exchangers such as controlled blocks or platforms to provide accurate temperature control of incubating samples from ~~4oC to 100oC~~ 4°C to 100°C; this is in addition to or in place of the station thermocontrollers. -

Please replace the paragraph beginning on page 59, line 10 with the following amended paragraph:

- A fluorescent reporter system was designed for quantifying intein cyclization. GFP was split at the loop 3 junction and the translational order of the N and C-terminal fragments were reversed (Figure 12A). The termini were held together by a glycine-serine linker. In some constructs, one-half of the myc epitope was fused onto either side of ~~theloop~~ the loop 3 junction (Figure 12A). The resulting GFP molecules were positioned with an intein scaffold comprising either wild-type or a mutant intein (Figure 12C). -

Please replace the paragraph beginning on page 59, line 17 with the following amended paragraph:

- Mutant intein sequences obtained using PCR mutagenesis were screened for activity by FACS sorting for increases in fluorescence.. Western blot analysis of several other mutants is shown in Figure 13. In Figure 13, several of the mutants had cyclization efficiencies greater than ~~theparental staring~~ the parental starting intein, J3. -

Please replace the paragraph beginning on page 59, line 26 with the following amended paragraph:

- To test the effects of a fixed proline in a cyclic 7mer, the conformation space of the 7mer cyclic peptide RGDGWS, containing two flexible glycines was compared with that of cyclic RGPGWS using ~~quenced~~ quenched molecular dynamics calculations (O'Connor, et al., (1992) J. Med. Chem., 35:2870-81; Mackay, et al., (1989) "The role of energy minimization in simulation ~~stragegies~~ strategies of biomolecular systems", In Prediction of Protein Structure and the Principles of Protein Conformation, Fasman, G., ed., New York, ~~Plemum~~Plenum Press, pp. 317-358. -

Please replace the paragraph beginning on page 60, line 1 with the following amended paragraph:

- An example of the cluster graph of the lowest energy conformers for each peptide is shown in Figures ~~15~~16 and ~~16~~17. The root mean square deviation (RMSD, Å) is coded by color, with very similar conformers (RMSD < Å) in yellow, still highly similar conformers (RMSD between 1-2 Å) in white, similar conformers (RMSD between 2-3 Å) in blue, less similar conformers (RMSD between 3-4 Å) in red, and dissimilar conformers in black (not shown). -

Please replace the paragraph beginning on page 60, line 7 with the following amended paragraph:

- For the cyclic peptide SRGDGWS, shown in Figure ~~15~~16 (srgdgwsLowest5A.ps), there were 62 low energy conformers. There was one family of very similar conformers (yellow square at bottom left) and two families of quite similar conformers in yellow/white, one roughly in the middle of the graph, and one (with only moderately similar conformers) near the top right corner. These comprised approximately 20 of the 62 conformers. The rest of the low energy conformers were not very similar to each other, and much of the graph is red or black. Backbone overlaid conformers from most similar family, No. 1, are shown at the lower left. In the lower middle, is family No. 2. ~~these~~These conformers, when overlaid are clearly not similar. Conformers in family No. 3 (lower right)[,] are rather heterogeneous, although not as much as those from the red and black regions of the graph. -

Please replace the paragraph beginning on page 60, line 17 with the following amended paragraph:

- For the cyclic peptide SRGPGWS, representing the substitution of ~~pro for asp~~ Pro for Asp 4, the graph of the lowest energy conformers looks quite different (Figure ~~16~~17; srgpgwsLowest5B.ps). There is a much larger family of very similar conformers (lower left of graph, family No. 1, conformers 1-26). Family No. 2 also has very similar conformers, although they are all different from family No. 1. Even family No. 3, representing over two thirds of all low energy conformers (frames 1-59) contains conformers that are similar enough to give a blurred donut appearance. Thus, substitution of a single pro for another residue (asp in this case) clearly freezes out two additional families of conformers. As this

peptide has two glycines, the effect of proline on conformational narrowing of cyclic peptides with 1 or 0 glycines may be more profound. –

Please replace the paragraph beginning after the Abstract with the following amended paragraph:

- ~~The present invention relates ti methods and compositions utilizing inteins to generated libraries of cyclic peptides *in vivo*.~~ The compositions relate to retroviral vectors encoding intein containing polypeptides which, when expressed in an eukaryotic cell, are capable of generating cyclic peptides. The retroviral vectors are used in methods for producing cyclic peptides and for identifying peptides capable of altering cellular phenotypes.